Practical application of Agrobacterium-mediated co-transformation using negative selection marker gene (codA) to produce transgenic rice.

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This study was conducted to investigate the practical application of co-transformation system for producing marker-free plants of rice. Scutella-derived embryogenic calli of rice (Cv ; Ilmi-byeo) were co-transformed with Agrobacterium tumefecie (Gv3101) harbored with ABF3 and codA in independent vectors, co-cultured on N6 basal medium containing acetosyringone under the dark at 25°C, followed by selected and regenerated under the presence of 50mg/L hygromycin. ABF3 gene was known as abiotic stress resistant gene identified from Arabidopsis. CodA gene, which convert non cyto-toxic material, 5-FC, to toxic 5-FU, has been widely used in bacteria, yeast and animal research to make mutations in specific genes, recently in plants for producing marker free transformants as negative selection marker. Total eight sets of co-transformation were conducted using Agrobacterium. Callus selection and shoot regeneration efficiency against hygromycin were 10.4% and 1.4%, respectively. However an amount of regenerated shoot were withered by unidentified factors while shoots were emerged followed by rooting. For the reason, shoot regeneration efficiency was decreased to 0.9%. It was considered that Agrobacterium strain is one of the reason at the efficiency. Thus to compare between Agrobacterium strains, it will be performed with another one. Totally, thirty one of plants which resist hygromycin were obtained. And plants were being analyzed by PCR and Southern blot. To certify the function of codA gene, T1 seeds will be germinated with medium containing 5-FC or 5-FU.

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